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The Set3 Complex Antagonizes the MYST Acetyltransferase Esa1 in the DNA Damage Response

Ana Lilia Torres-Machorro, Lauren G. Clark,* Christie S. Chang,* Lorraine Pillus

Section of Molecular Biology, Division of Biological Sciences, University of California, San Diego, UC San Diego Moores Cancer Center, La Jolla, California, USA

Acetylation is a dynamic posttranslational modification that contributes to chromatin-regulated processes, including DNA replication, repair, recombination, and gene expression. Acetylation is controlled by complexes containing opposing lysine and histone acetyltransferase (KAT and HAT) and deacetylase (KDAC and HDAC) activities. The essential MYST family Esa1 KAT acetylates core histones and many nonhistone substrates. Phenotypes of *esa1* mutants include transcriptional silencing and activation defects, impaired growth at high temperatures, and sensitivity to DNA damage. The KDAC Rpd3 was previously identified as an activity opposing Esa1, as its deletion suppresses growth and silencing defects of *esa1* mutants. However, loss of Rpd3 does not suppress *esa1* DNA damage sensitivity. In this work, we identified Hos2 as a KDAC counteracting *ESA1* in the damage response. Deletion of *HOS2* resulted in changes of *esa1*'s transcriptional response upon damage. Further, loss of *HOS2* or components of the Set3 complex (Set3C) in which it acts specifically suppressed damage sensitivity and restored *esa1* histone H4 acetylation. This rescue was mediated via loss of either Set3C integrity or of its binding to dimethylated histone H3K4. Our results thus add new insight into the interactions of an essential MYST acetyltransferase with diverse deacetylases to respond specifically to environmental and physiological challenges.

Chromatin regulates gene expression, recombination, and replication and DNA damage repair (1, 2). It is subject to multiple posttranslational modifications (3), including lysine acetylation, a dynamic modification that is established by lysine and histone acetyltransferases (KATs and HATs) and reversed by lysine and histone deacetylases (KDACs and HDACs). Acetylation partially neutralizes the basic charge of histone tails, relaxing nucleosome compactness. It also creates binding sites for proteins containing bromodomains and has been linked to an open chromatin conformation (4).

The Esa1 acetyltransferase acts in two different complexes, piccolo and NuA4 (5), in which it preferentially acetylates histones H4 and H2A and the histone variant Htz1 (6–11). Additionally, it acts on nonhistone substrates, such as the NuA4 subunits Epl1 and Yng2 (7, 12), the autophagy protein Atg3 (13), the RNA processing protein Nab3 (14, 15), and nearly 200 other proteins (12, 15, 16). Notably, the Tip60 human ortholog of Esa1 has been linked to multiple human diseases (17–19), thus increasing the relevance of gaining a deeper understanding of Esa1 functions.

ESA1 is an essential gene contributing to transcriptional regulation in response to growth stimuli that has been most extensively studied with conditional alleles (6–8, 20). Hypomorphic *esa1* strains are temperature sensitive and have defects in progression through the G_2/M phase of the cell cycle and transcriptional regulation, including failure to silence ribosomal DNA (rDNA) and telomere proximal genes (6, 21). *ESA1* mutants are also defective in repairing DNA damage (22, 23).

DNA damage results from many environmental factors, such as UV and gamma irradiation or heavy metal toxins; it can also be introduced by intrinsic factors such as reactive oxygen species, DNA replication, and others (24–26). Among the many types of damage, DNA double-stranded breaks (DSBs) are deleterious lesions which if unrepaired can lead to mutation, cell death, and cancer in metazoans. Cells ordinarily respond to DNA damage by signal transduction cascades that lead to pauses in the cell cycle to allow repair, wide changes in gene expression, and direct action at the breaks, promoting rapid ligation of the broken DNA ends (27–30). Mutations can affect any and all stages of the repair processes.

Esa1 contributes to multiple aspects of the DNA damage response by regulating gene expression (31, 32) and by its direct recruitment to DSBs, where it promotes signaling to repair the breaks (22, 33). Multiple suppressors of *esa1* phenotypes have been identified (14, 23, 34–36); however, suppression of DNA repair defects of *esa1* has not yet been fully explored.

In a search to understand repair in *esa1* mutants, we have now identified a role for an opposing deacetylase, Hos2. Hos2 is a class I KDAC necessary for induction of gene expression, perhaps by creating a permissive chromatin state for multiple rounds of transcription (37). More recently, however, it has been suggested that its activating role could be closely tied to repressing noncoding RNAs (ncRNAs) that overlap many Hos2-regulated genes (38). Hos2 is a component of the Set3 complex (Set3C), which includes the sirtuin deacetylase Hst1 (39). Set3C is important in regulating gene induction during the stress response, including changes in carbon sources (38), nitrogen starvation (39), and DNA damage

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Address correspondence to Lorraine Pillus, lpillus@ucsd.edu.
* Present address: Lauren G. Clark, Department of Chemical and Biological Engineering, Northwestern University, Evanston, Illinois, USA; Christie S. Chang, Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, New Jersey, USA.
A.L.T.-M. and L.G.C. contributed equally to this article.

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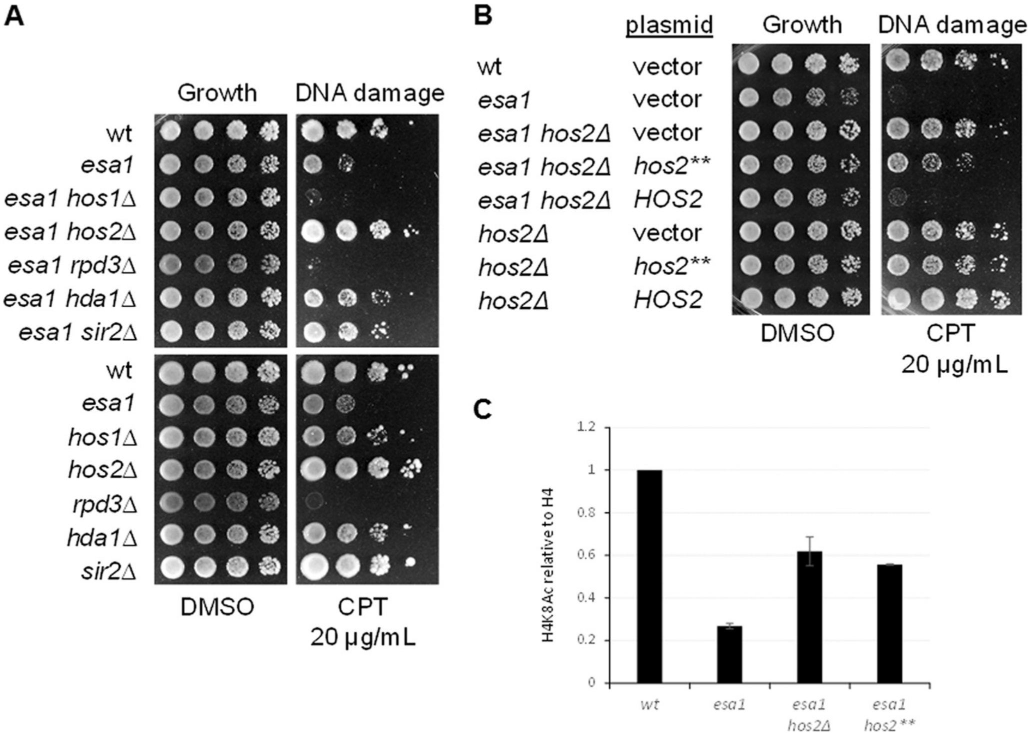

FIG 1 Deletion of the histone deacetylase encoded by *HOS2* suppressed the DNA damage sensitivity of *esa1*. (A) Deletion of *HOS2* rescued *esa1* DNA damage sensitivity, whereas deletion of *HOS1* and *RPD3* did not. Deletion of *SIR2* and *HDA1* partially rescued *esa1*. Shown are serial dilutions of wild-type (wt) (LPY5), *esa1-414* (LPY4774), *esa1-414 hos1*Δ (LPY13712), *esa1-414 hos2*Δ (LPY13585), *esa1-414 rpd3*Δ (LPY12156), *esa1-414 hda1*Δ (LPY13478), *esa1-414 sir2*Δ (LPY11279) strains (top) and wild type (LPY5), *esa1-414* (LPY4774), *hos1*Δ (LPY13706), *hos2*Δ (LPY13583), *rpd3*Δ (LPY12154), *hda1*Δ (LPY13472), and *sir2*Δ (LPY11) strains (bottom). Figure S1A in the supplemental material shows the phenotype of the same strains grown at 37°C. Note that some of these interactions overlap results from a genomewide study (7), yet others are distinct, an effect that we find is due to strain background differences. See Fig. S1B to D for more details. (B) Loss of the deacetylase activity of Hos2 was important for *esa1* suppression. Strains in panel A were transformed with vector (pLP60), *HOS2* (pLP2567), or *hos2-H194A,H196A* (*hos2***; pLP2569) and tested for DNA damage sensitivity. CPT and DMSO plates were prepared without histidine to maintain the plasmid. (C) Suppression in *esa1 hos2*Δ strains transformed with a vector and with *hos2*** correlated with increased histone H4K8 acetylation. Quantification of H4K8Ac levels relative to histone H4 was performed with ImageQuant 5.2 (Molecular Dynamics). The histogram peak function was applied to correct for background. Representative immunoblots are shown in Fig. S1D.

(31). Set3C binds to the histone mark H3K4me2 (40), generally found in the 5′ region of the open reading frames (ORFs); however it can also be enriched in promoter regions of some genes, replacing H3K4me3 (38).

In this work, we report that Hos2 is the relevant activity opposing Esa1 in DNA damage repair. We found that *esa1* had defects in transcriptional induction of DNA damage-regulated genes that were attenuated upon deletion of *HOS2*. Suppression by *hos2*Δ was in the context of Set3C, because deletion of other complex components also suppressed the DNA damage sensitivity of *esa1* mutants. Loss of Set3C recruitment to the H3K4me2 mark rescued *esa1*'s repair defects, supporting the concept that suppression was mediated through the DNA damage transcriptional response.

MATERIALS AND METHODS

Yeast strains and plasmids. Strains, plasmids and oligonucleotides are listed in Tables S1 to S3 in the supplemental material. The *esa1-414* and *esa1-531* alleles have been previously characterized (6, 7). Both alleles are sensitive to DNA damage; however, *esa1-531* is more defective than *esa1-414* at 30°C, allowing isolation of damage effects from those introduced by temperature stress. The *hst1*Δ*2*::*LEU2* (LPY18275) disruption was engineered into a wild-type BY strain (41). All other mutations were null alleles constructed using standard methods and backcrossed prior to use. Histone mutant strains had chromosomal deletions for both *HHF-HHT* loci and initially contained pJH33 (*HTA HTB HHF2 HHT2 URA3 CEN*) (42); these were transformed with *TRP1* plasmids carrying relevant H4 (*HHF2*) mutations. The plasmid pJH33 was selected against by growth on 5-fluoroorotic acid (5-FOA). The catalytic mutant *hos2-H195A,H196A* was constructed with primers listed in Table S3. Strains were grown at 30°C in yeast extract-peptone-dextrose medium plus adenine (YPAD) or dropout medium for selection.

Growth dilution assays, silencing assays, and flow cytometry. Unless otherwise noted, all dilution assays represent 5-fold serial dilutions, starting from an A_{600} of 0.5 after growth to saturation in YPAD. Growth and silencing assays were performed at 30°C as described previously (43, 44). For rDNA silencing, strains were grown in synthetic complete (SC) medium lacking adenine (Ade) and Arg (SC−Ade−Arg) to saturation, normalized as described above, and plated on SC−Ade−Arg and SC−Ade−Arg containing 32 µg/ml of canavanine. Telomeric silencing assays were conducted with plating on SC and SC with 0.1% 5-FOA. Camptothecin (CPT) sensitivity was assayed using CPT in dimethyl sulfoxide (DMSO) added to plates buffered with 100 mM potassium phosphate (pH 7.5) to maintain maximal drug activity (45). Growth control plates contained equal concentrations of DMSO and phosphate buffer. Images were captured after 2 to 6 days. Cells were processed for flow cytometry as described previously (14) and analyzed with Accuri (BD) after sonication.

Protein immunoblotting. Whole-cell extracts were prepared from cells grown to an A_{600} of 0.8 to 1.0 at 30°C in YPAD. For DNA damage, cells were grown to an A_{600} of 0.5 and were exposed for 90 min to hy-

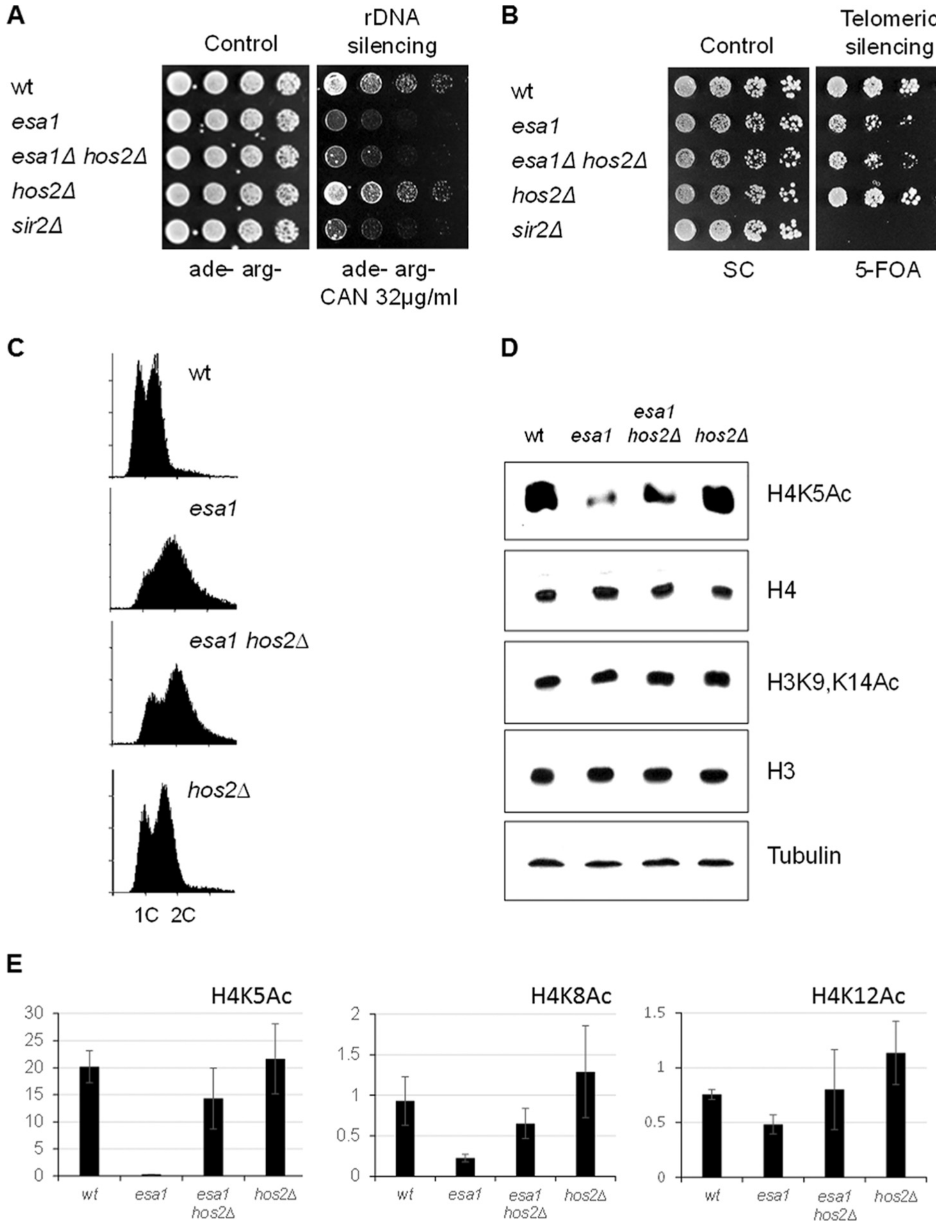

FIG 2 Deleting *HOS2* improved histone H4 acetylation in *esa1* but did not restore silencing and cell cycle regulation. (A) The *esa1 hos2*Δ strain is defective for rDNA silencing. The wild-type (LPY4908), *esa1-414* (LPY4912), *esa1-414 hos2*Δ (LPY18074), *hos2*Δ (LPY18073), and *sir2*Δ (LPY5015) strains carry the *rDNA*::*ADE2-CAN1* reporter. Defective rDNA silencing leads to expression of *CAN1* and sensitivity to canavanine. (B) The *esa1 hos2*Δ strain had telomeric silencing defects. The wild-type (LPY4916), *esa1-414* (LPY13520), *esa1-414 hos2*Δ (LPY18070), *hos2*Δ (LPY18071), and *sir2*Δ (LPY5034) strains carry the *TELVR*::*URA3* reporter. Defective telomeric silencing results in 5-FOA sensitivity. (C) Cell cycle profiles showed a significant G_2/M delay in cell cycle progression at 30°C in *esa1-531* (LPY14757) cells that was modestly improved in the *esa1-531 hos2*Δ (LPY14761) strain. Control strains were the wild-type (LPY6497) and *hos2*Δ (LPY14577) strains. (D) Deletion of *HOS2* increased acetylation of histone H4K5 in *esa1* mutants. H3K9 and K14 acetylation was unaffected by mutation of *ESA1* or *hos2*Δ. Whole-cell protein lysates from strains in panel C were immunoblotted as noted. The experiment was also performed with *esa1-414* strains, with similar results. (E) Deletion of *HOS2* improved acetylation of other H4 lysines in *esa1* strains. Quantification of histone H4 acetylation at K5, K8, and K12 relative to histone levels was performed using two to four independent Western blots. ImageQuant 5.2 (Molecular Dynamics) and the histogram peak function were used as for Fig. 1. Representative immunoblots are shown in Fig. S2 in the supplemental material.

droxyurea at 0.2 M or to a carrier. Extracts were prepared as described previously (6) by vortexing cells with glass beads in phosphate-buffered saline (PBS) with protease inhibitors, denaturing in boiling sample loading buffer, and separating the insoluble pellet by centrifugation. Samples were separated on 18% SDS-polyacrylamide gels and transferred to 0.2-µm nitrocellulose. Primary antisera were anti-H4K5Ac (1:5,000 dilution; Serotec), anti-H4K8Ac (1:2,000; Serotec), anti-H4K12Ac (1:2,000; Active Motif), anti-H4K16Ac (1:2,000; Millipore), anti-H3K9 and -K14Ac (1:10,000; Upstate), anti-H3K14Ac (1:2,000; Upstate), anti-H4 (1:2,000; Active Motif 39269), and anti-H3ct (1:10,000; Millipore). The secondary reagent was horseradish peroxidase-conjugated goat anti-rabbit antibody (Promega, 1:10,000). Blots were quantified using the ImageQuant 5.2 program (Molecular Dynamics). The histogram peak function was applied to correct for background signal.

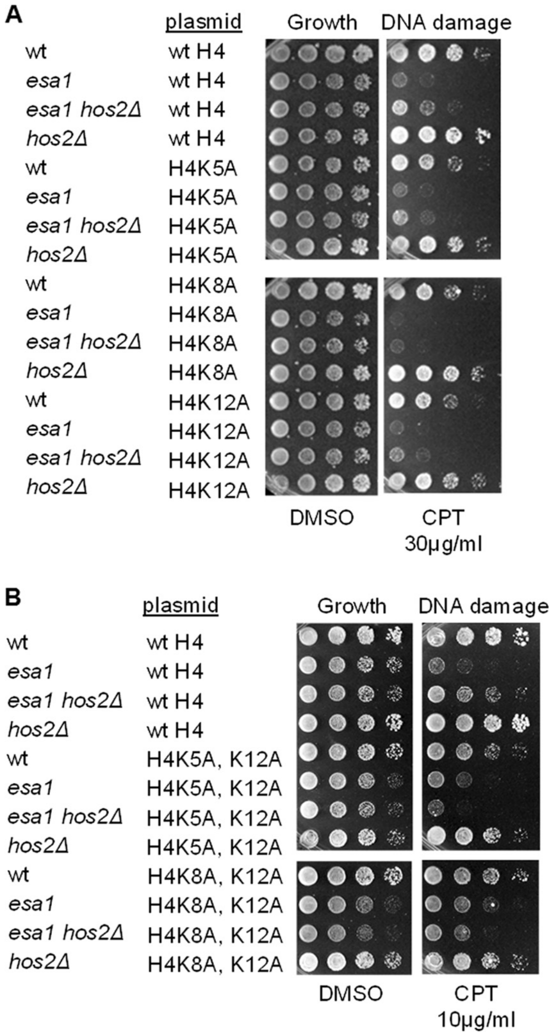

FIG 3 Suppression of *esa1* by *hos2*Δ was dependent upon both individual and combined H4K5, K8, and K12 histone target residues. (A) Mutation of single H4K5, K8, or K12 residues to alanine did not fully disrupt suppression of *esa1* DNA damage sensitivity by *hos2*Δ. Serial dilutions of strains with histone genes deleted and restored on plasmids included wild-type (LPY14161), *esa1-414* (LPY14163), *esa1-414 hos2*Δ (LPY15906), and *hos2*Δ strains expressing wild-type histones from a plasmid; wild-type (LPY13656), *esa1-414* (LPY13064), *esa1-414 hos2*Δ (LPY15911), and *hos2*Δ (LPY17911) strains expressing H4K5A from a plasmid; wild-type (LPY14162), *esa1-414* (LPY14164), *esa1-414 hos2*Δ (LPY15912), and *hos2*Δ (LPY17912) strains expressing H4K8A from a plasmid; and wild-type (LPY13060), *esa1-414* (LPY13063), *esa1-414 hos2*Δ (LPY15913), and *hos2*Δ (LPY17913) strains expressing H4K12A from a plasmid. Fivefold dilutions were plated as indicated. Note that suppression of *esa1* DNA damage sensitivity was not as strong as in Fig. 1A, likely because the histone mutant background has altered histone dosage that can affect sensitivity (72). (B) The *esa1 hos2*Δ strains containing combined H4K5A and K12A and H4K8A and K12A mutations did not suppress *esa1* DNA damage sensitivity and in some cases appeared more sensitive to damage. Wild-type (LPY19424), *esa1-414* (LPY19406), *esa1-414 hos2*Δ (LPY19425) and *hos2*Δ (LPY19426) strains expressing H4K5A and K12A and wild-type (LPY19420), *esa1-414* (LPY19421), *esa1-414 hos2*Δ (LPY19422), and *hos2*Δ (LPY19423) strains expressing H4K8A and K12A were compared to strains containing wild-type histones.

RNA extraction and RT-PCR. Strains were grown in 50 ml of 100 mM phosphate-buffered YPAD (pH 7.5) at 30°C. At an A_{600} of 0.4 to 0.5, cultures were split and treated with CPT (20 μg/ml) or a DMSO carrier. After 90 min at 30°C, RNA was extracted using the hot acid-phenol method (46), except that harvested cells were resuspended in sodium acetate buffer (50 mM sodium acetate [pH 5.3], 10 mM EDTA). After extraction, RNA was treated with the TURBO DNA-*free* kit (Ambion) and reverse transcribed using TaqMan reverse transcription reagents (Applied Biosystems) with random hexamer priming. The cDNA was then diluted 10-fold and analyzed by real-time PCR with a SYBR green PCR mix (Anaspec) on a DNA Engine Opticon2 (MJ Research). Oligonucleotides are listed in Table S3 in the supplemental material. Data shown in Fig. 4 are the averages of three separate RNA extractions analyzed in triplicate.

RESULTS

Genetic suppression of conditional alleles of *ESA1* has provided insight into its regulation and roles in different cellular pathways. Deletion of *RPD3*, which encodes a global KDAC, suppresses the temperature and silencing defects of *esa1* strains; however, *esa1 rpd3*Δ cells remain sensitive to DNA damage (23), suggesting the involvement of a different enzyme in opposing Esa1's function during response to DNA damage.

The DNA damage sensitivity of *esa1* can be suppressed by deletion of *HOS2*. As KDACs oppose acetylation established by KATs, we hypothesized that a KDAC other than Rpd3 could suppress the DNA damage sensitivity of *esa1*. Initial candidates tested included Hos1 and Hos2 (similar in sequence to Rpd3 and classified as type I KDACs), the type II KDAC Hda1 (47), and the sirtuin Sir2 (48). Growth of double *esa1* mutants in combination with deletions of the candidate KDACs was tested by challenge with the DSB-inducing drug camptothecin (CPT) (Fig. 1). The *RPD3* and *HOS1* deletions increased sensitivity, whereas the *HOS2*, *HDA1*, and *SIR2* deletions suppressed *esa1* (Fig. 1A). As *hos2*Δ promoted the strongest growth, we focused on characterizing this suppression.

To investigate whether suppression of *esa1* by *hos2*Δ was dependent on loss of deacetylase activity, *esa1 hos2*Δ strains were transformed with *HOS2*, its catalytic mutant *hos2-H195A,H196A* (37), or a vector control. Transformants were tested for sensitivity to CPT. As shown in Fig. 1B, wild-type *HOS2* expression in the *esa1 hos2*Δ strain mirrored the DNA damage sensitivity of the *esa1* strain, whereas the *esa1 hos2*Δ strain transformed with *hos2-H195A,H196A* had decreased sensitivity, suggesting that loss of Hos2's catalytic activity was important for suppression. Both *esa1 hos2*Δ strains transformed with vector and with *hos2-H195A,H196A* also showed increased H4 acetylation levels relative to the *esa1* strain transformed with vector (Fig. 1C). In contrast to *hos2*Δ, expression of catalytically dead Hos2 would not likely disrupt the integrity of the Set3 complex. This explains the partial suppression of *esa1* when *esa1 hos2*Δ was transformed with *hos2-H195A,H196A*, as loss of other subunits of Set3C also have a role in suppressing *esa1* (see below).

***hos2*Δ suppressed low histone H4 acetylation of *esa1*.** In addition to DNA damage sensitivity, *ESA1* mutant strains are also characterized by defects in transcriptional silencing and progression through the cell cycle and by low levels of histone H4 acetylation (6, 21). Deletion of *HOS2* was tested for suppression of these phenotypes.

Three transcriptionally silenced regions in *Saccharomyces cerevisiae* are the ribosomal DNA (rDNA) repeats, telomeres, and the silent mating-type loci (49). *ESA1* mutants are defective in silencing the rDNA and telomeres (20) when assayed with reporter strains. The reporter for the rDNA has an *ADE2-CAN1* cassette inserted in one of the rDNA repeats in chromosome XII (50). Defects in silencing lead to expression of the *CAN1* gene,

TABLE 1 Characteristics of DNA damage-regulated genes tested[a]

Gene	H3K4me2	ncRNA	Expression in: *hos2Δ*	Expression in: *set3Δ*	Role
HUG1	Promoter	Meiotic ORF (AS)	—	—*	Involved in Mec1 checkpoint
ERG5	Promoter and 5′ ORF	XUT (AS) promoter, CUT (S) promoter		—*	Oxidation reduction and lipid metabolism
GRE2	Promoter and ORF	ncRNA (AS) whole ORF	—*	—*	Methylbutanal and glyoxal reductase

[a] The H3K4me2 column indicates if dimethylation of H3K4 was identified in a previous global survey (56) and its localization within the gene body (*Saccharomyces* Genome Database). The ncRNA column specifies the type, localization, and orientation, sense (S) or antisense (AS), of ncRNAs overlapping the genes tested (54, 55, 57, 58). The *hos2Δ* and *set3Δ* columns show if expression was downregulated (—) in the corresponding null strains (60) and whether the change was statistically significant (*).

which encodes an arginine permease. Canavanine is a toxic arginine analog that is imported into cells only when *CAN1* is expressed. When incorporated into proteins, it leads to reduced growth due to defects in protein folding. The telomeric reporter consists of a *URA3* gene inserted on chromosome VR. Its expression inhibits growth on 5-FOA, which is toxic for cells expressing *URA3* (50). The *esa1 hos2Δ* strain showed growth patterns similar to those of *esa1* with both silencing reporters. Thus, *HOS2* deletion could not suppress the silencing defects of *esa1* (Fig. 2A and B).

As *esa1* mutants have defects in progression through G_2/M (6), cell cycle profiles were evaluated by flow cytometry. The *esa1 hos2Δ* strain showed a delay in progression through G_2/M similar to that of the *esa1* strain; thus, *hos2Δ* cannot restore cell cycle regulation of *esa1* (Fig. 2C).

Conditional *esa1* mutants have low levels of histone H4 acetylation, especially for histone H4K5, a major target for Esa1. To test if deletion of *HOS2* suppressed the global acetylation defect of *esa1* strains, the status of Esa1 target lysines in histone H4 was assessed by immunoblotting. Deletion of *HOS2* suppressed the low histone H4K5 acetylation levels of the *esa1* strain (Fig. 2D), whereas H3 acetylation remained unchanged. Acetylation of H4K8 and H4K12 was also improved in the *esa1 hos2Δ* strain relative to that of the *esa1* strain (Fig. 2E). To determine if DNA damage affected global histone acetylation levels, immunoblots were analyzed for samples after DNA damage was induced. The results were similar to those for cells without damage induction. The *esa1 hos2Δ* strain had improved acetylation of histone H4K5 compared to that of the *esa1* strain (Fig. 2E; see also Fig. S2 in the supplemental material). Acetylation of H4K16 and H3K14 was unaffected by either *hos2Δ* or DNA damage in the *esa1* background. The suppression of the *esa1* global acetylation defect at H4K5 by *hos2Δ* thus appeared to be independent of DNA damage.

Loss of *HOS2* suppressed *esa1* through lysines 5, 8, and 12 of histone H4. Because suppression of temperature sensitivity of *esa1* by deletion of *RPD3* is dependent on lysine 12 of histone H4 (23), we hypothesized that suppression of *esa1* by *hos2Δ* could be mediated through one of the histone H4 lysines. To test this idea, we constructed *esa1* and *esa1 hos2Δ* strains in combination with a series of histone mutants replacing lysines of histone H4 with alanines as proxies of nonmodifiable residues. As shown in Fig. 3A, the *esa1 hos2Δ* strains in combination with single H4K5A, H4K8A, or H4K12A mutants were sicker when grown on CPT than the strains expressing wild-type histones, although they still had improved growth relative to that of *esa1* strains expressing the same histone mutants.

Lysines 5, 8, and 12 of H4 were previously reported to perform overlapping roles *in vivo* and to be modified by the same complexes (51). We hypothesized that the acetylation of multiple H4 lysines could contribute cooperatively to suppression of *esa1* by *hos2Δ*. Strains containing combined H4 lysine mutants were tested. As shown in Fig. 3B, the *esa1 hos2Δ* H4K5A, K12A and *esa1 hos2Δ* H4K8A, K12A strains were more sensitive to CPT than the corresponding *esa1* strains, suggesting that suppression of *esa1* by *HOS2* deletion required a combination of modifications of H4 lysines 5, 8, and 12.

Deletion of *HOS2* modulated the transcriptional response of *esa1* cells upon DNA damage. Esa1 is important during DNA damage repair in at least two different pathways. The first is through modulation of gene expression (31, 32), and the second is through its direct recruitment to sites of damage to carry out specific modifications promoting repair signaling at the break sites (22, 52). Because Hos2 was reported to influence induction of gene expression under stress conditions (31, 38) but not to be recruited to sites occupied by Esa1 at the DNA double-strand breaks (7), we analyzed the transcriptional response to DNA damage in *esa1* and *esa1 hos2Δ* strains. Multiple independent genome-wide data sets were used to select candidates for analysis (53–56). As the Hos2-containing Set3C is recruited to H3K4me2-marked genomic areas and has a role in regulation of ncRNA expression, we identified genes with this histone mark and ncRNAs (54–58). Selected genes were reported to be induced upon methyl methanesulfonate (MMS)-induced DNA damage (59) and had global expression changes in *hos2Δ* or *set3Δ* mutants, even in the absence of damage (60). Table 1 summarizes published data for the genes tested. For example, the *HUG1* locus has the H3K4me2 mark in its promoter region and is downregulated in *hos2Δ* and *set3Δ* strains.

To test if transcripts of the selected genes are regulated by Esa1, cells from wild-type, *esa1*, *esa1 hos2Δ*, and *hos2Δ* strains were treated with CPT or with the vehicle control DMSO for 90 min. RNA was purified and cDNA for each sample was used to quantify the expression levels of candidate genes (Fig. 4).

Expression of *HUG1* and *GRE2* was increased in the wild-type strain treated with CPT, as previously reported for other damage-inducing drugs (Fig. 4A) (59). Although repressed by MMS-induced DNA damage (59), *ERG5* expression was induced by CPT (Fig. 4A). This difference may reflect distinct effects on gene expression for MMS and CPT, for which mechanisms of damage response are known to be distinct (61, 62).

Mutant *esa1* cells had lower levels of expression in DMSO than wild-type for *HUG1* and *ERG5*. Both genes' expression upon damage was also lower than wild-type in *esa1* cells. In contrast, *GRE2* expression in *esa1* cells had higher levels of expression than the wild type when treated with DMSO and CPT (Fig. 4A).

H3K4me2 marks are present at *HUG1*, *ERG5*, and *GRE2* (Table 1). *HUG1* and *ERG5* are also marked by H3K4me3 at the

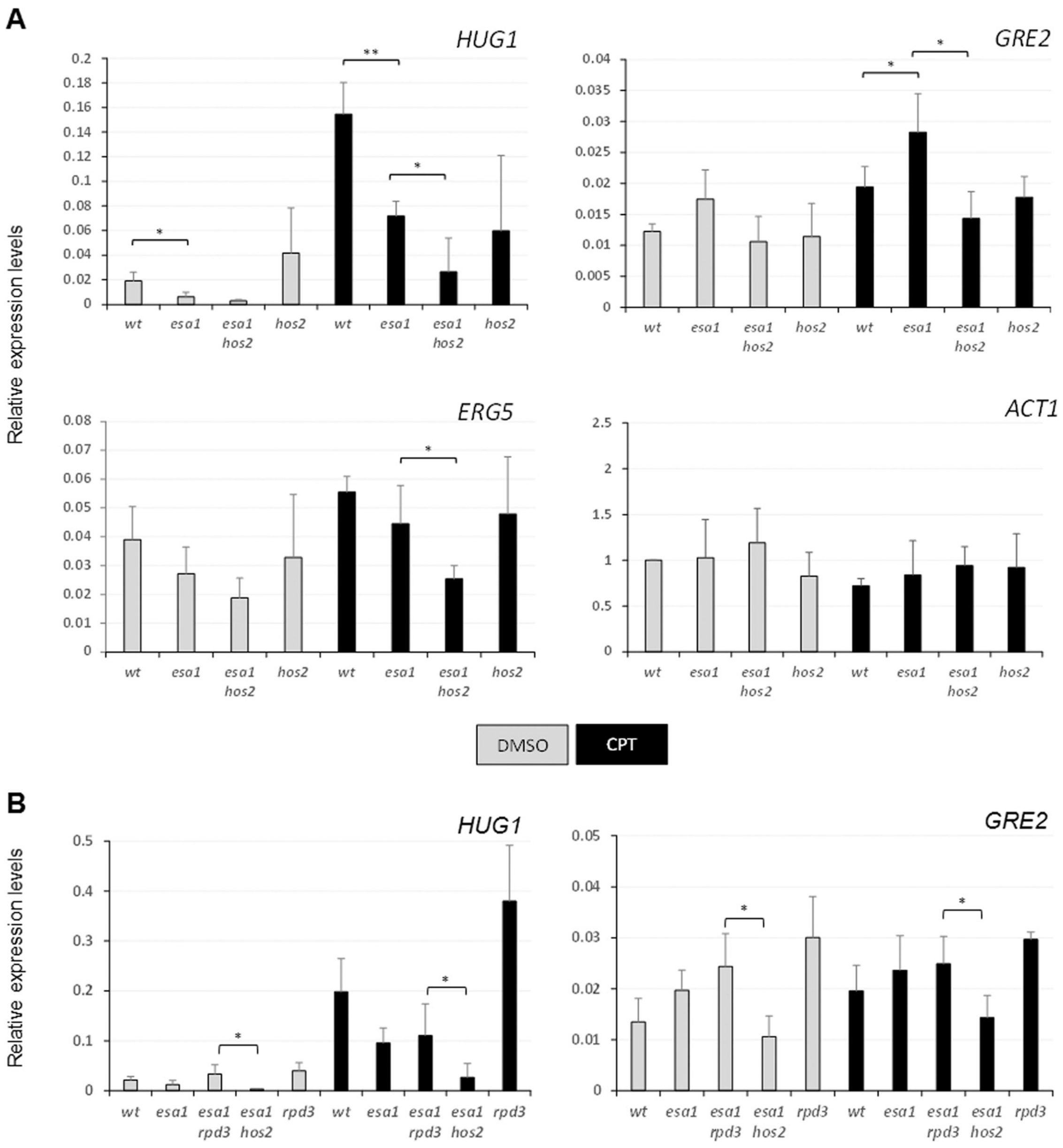

FIG 4 Expression of DNA damage response genes was aberrant in *esa1* cells. (A) Gene expression was analyzed in samples treated with DMSO or with 20 μg/ml of CPT for 90 min. The patterns of expression of the damage-activated genes *HUG1*, *GRE2*, and *ERG5* and the control gene *ACT1* are shown for wild-type (LPY6497), *esa1-531* (LPY14757), *esa1-531 hos2*Δ (LPY14761), and *hos2*Δ (LPY14577) strains. The expression values were normalized to *ACT1* expression. Three independent RNA samples were reverse transcribed and analyzed by qPCR with primers in Table S3 in the supplemental material. Student's *t* test was used to assess statistical significance, represented with asterisks as follows: *, $P < 0.05$, and **, for $P < 0.01$. (B) *HUG1* and *GRE2* expression levels upon damage were similar in *esa1* and *esa1 rpd3*Δ strains but distinct from that in the *esa1 hos2*Δ strain. Gene expression was analyzed in samples treated with DMSO or with 20 μg/ml of CPT for 90 min. The expression values were normalized to *ACT1* expression. Expression was analyzed in wild-type (LPY6496), *esa1-531* (LPY14757), *esa1-531 rpd3*Δ (LPY21450), *esa1-531 hos2*Δ (LPY14761), and *rpd3*Δ (LPY13426) strains.

promoter region, whereas *GRE2* completely lacks this mark (56). We hypothesize that the variable pattern of expression of *GRE2* in the *esa1* strain compared to *HUG1* and *ERG5* reflects the possibility that *GRE2* regulation is initially independent of the H3K4me3 mark present in *HUG1* and *ERG5*.

The above-described results show that gene expression of DNA damage-responsive genes in *esa1* cells is aberrant in DMSO- and CPT-treated samples. Expression for all three tested genes in the *esa1 hos2*Δ strain proved more uniform: lower than in wild-type and *esa1* cells treated with either DMSO or CPT (Fig. 4A). When comparing DMSO- and CPT-treated samples for *HUG1*, *GRE2*, and *ERG5*, we found that the *esa1 hos2*Δ strain showed small changes in expression, whereas an increase in gene expression upon damage was clear in *esa1* and wild-type cells.

As reported earlier, deletion of *RPD3* can also suppress some *esa1* mutant phenotypes through increased histone H4 acetylation, although not its DNA damage sensitivity (34). We hypothesized that the expression pattern in DNA damage-sensitive *esa1* *rpd3*Δ cells would be different than the pattern found in the damage-resistant *esa1 hos2*Δ cells. As shown in Fig. 4B, when cells were treated with DMSO or CPT, expression of *HUG1* and *GRE2* in the *esa1 rpd3*Δ strain was similar to that of the *esa1* strain, whereas in the *esa1 hos2*Δ strain, expression was low under all conditions relative to those in the wild-type, *esa1*, and *esa1 rpd3*Δ strains. This result shows that the *esa1 hos2*Δ expression pattern, although not fully equivalent to that of the wild type, is distinct from the pattern found in the damage-sensitive *esa1* and *esa1 rpd3*Δ strains, consistent with the *in vivo* suppression we observed.

Loss of the Set3 complex suppressed *esa1*. Hos2 is a centrally important component of Set3C, a complex with important roles in stress responses. The eponymous Set3 subunit has both PHD and SET domains (39). Other subunits of the complex include Snt1, Sif2, Cpr1, and Hos4, and catalytic subunits Hos2 and the class III KDAC sirtuin Hst1 (Fig. 5A). Hst1 is not a dedicated complex member, but it is also found in the Sum1 complex (Sum1C, containing Sum1, Rfm1, and Hst1), which functions mitotically to silence meiotic and sporulation genes (Fig. 5A) (63).

To examine if suppression of *esa1* DNA damage sensitivity was mediated by the Set3C, we constructed double mutants combining *esa1* with deletions of genes encoding Set3C components. Loss of any Set3C subunit tested suppressed *esa1*'s DNA damage sensitivity (Fig. 5B). The suppression was not uniform: *HOS4*, *SET3*, *HOS2*, and *HST1* deletions were more effective than deletions of *SNT1* or *SIF2*.

Loss of Set3 and Hos2 leads to disassembly of the complex (39), whereas loss of Hos4 results in further loss of Hst1. Our results suggest that both Hos2 and Hst1 KDACs are important in opposing Esa1 during the DNA damage response. However, suppression of *esa1* by *hst1*Δ was clearly mediated through Set3C and not through Sum1C, as loss of the Sum1 subunit did not suppress *esa1* (Fig. 5C).

Impaired binding of Set3C to H3K4me2 suppressed *esa1*. As Set3C binds H3K4me2 to influence induction of gene expression under stress conditions, we asked if suppression of DNA damage sensitivity was dependent on H3K4 methylation by deleting *SET1*, which encodes the H3K4 methyltransferase of the COMPASS complex (64). The *esa1 set1*Δ strain was extremely sick (Fig. 6A), with slow growth at 30°C and sensitivity to DMSO. We reasoned that reduced viability could be due to complete loss of H3K4 methylation. Consistent with this idea, the H3K4A mutant alone was very sensitive to damage in the *esa1* background, and deletion of *HOS2* could not suppress this phenotype (Fig. 6B).

Loss of COMPASS subunits differentially affects di- or trimethylation of H3K4 (reviewed in reference 65). For example, deletion of *CPS25* (*SDC1*) or *CPS60* (*BRE2*) promotes loss of H3K4me3 and diminished levels of H3K4me2, whereas deletion of *CPS40* (*SPP1*) is characterized by very low H3K4me3 levels (66–68). We considered the hypothesis that if H3K4me2 was reduced by deletion of *CPS25* or *CPS60*, Set3C regulation would be impaired in *esa1*, perhaps promoting resistance to DNA damage. However, the double *esa1 cps25*Δ and *esa1 cps60*Δ mutants were very sick and extremely sensitive to DNA damage (Fig. 6C). In contrast, the *esa1 cps40*Δ strain, which should only affect H3K4me3, grew comparably to the *esa1* single mutant (Fig. 6C), suggesting that H3K4me3 is not as critical as H3K4me2 in *esa1* cells. This result supports a previous report that specific loss of H3K4me3 had no significant impact on gene expression in wild-type cells, whereas simultaneous loss of H3K4me2 and H3K4me3 led to greater changes in gene expression (68). We further tested if *hos2*Δ could suppress *esa1* when H3K4me2 and H3K4me3 were impaired by deletion of *CPS25*. The triple *esa1 hos2*Δ *cps40*Δ mutant was also used as a control for specific loss of H3K4me3 that would have little effect on H3K4me2. Figure 6D shows that *hos2*Δ did not suppress *esa1* when *CPS25* or *CPS40* was also deleted. Thus, deletion of COMPASS subunits proved insufficient to test the role of H3K4me2 in suppression of *esa1* by *hos2*Δ for two reasons: because H3K4 methylation influences gene expression through

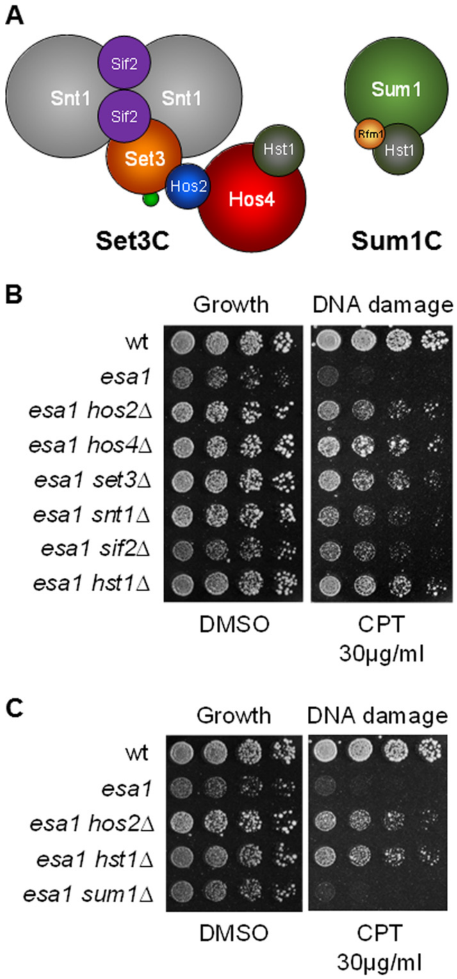

FIG 5 Deletion of Set3C subunits suppressed DNA damage sensitivity of *esa1*. (A) Subunit composition of Sum1C and Set3C (with data from reference 39). The relative size of the subunits is drawn to scale. The small green circle in Set3C represents the Cpr1 subunit. (B) Deletion of each Set3C subunit tested suppressed *esa1* sensitivity to CPT. Wild-type (LPY6497), *esa1-531* (LPY14757), *esa1-531 hos2*Δ (LPY14761), *esa1-531 hos4*Δ (LPY15865), *esa1-531 set3*Δ (LPY15869), *esa1-531 snt1*Δ (LPY15867), *esa1-531 sif2*Δ (LPY15863), and *esa1-531 hst1*Δ*2* (LPY18266) strains were tested. (C) Suppression of *esa1* DNA damage sensitivity was specific to Set3C. Deletion of *SUM1* had no effect on *esa1* sensitivity to CPT. Loss of *HST1* was comparable to *hos2*Δ for suppression of *esa1* DNA damage sensitivity. Wild-type (LPY6497), *esa1-531* (LPY14757), *esa1-531 hos2*Δ (LPY14761), *esa1-531 hst1*Δ*2* (LPY18266), and *esa1-531 sum1*Δ (LPY17505) strains were compared. Figure S3 in the supplemental material includes single mutant controls for Set3C subunits.

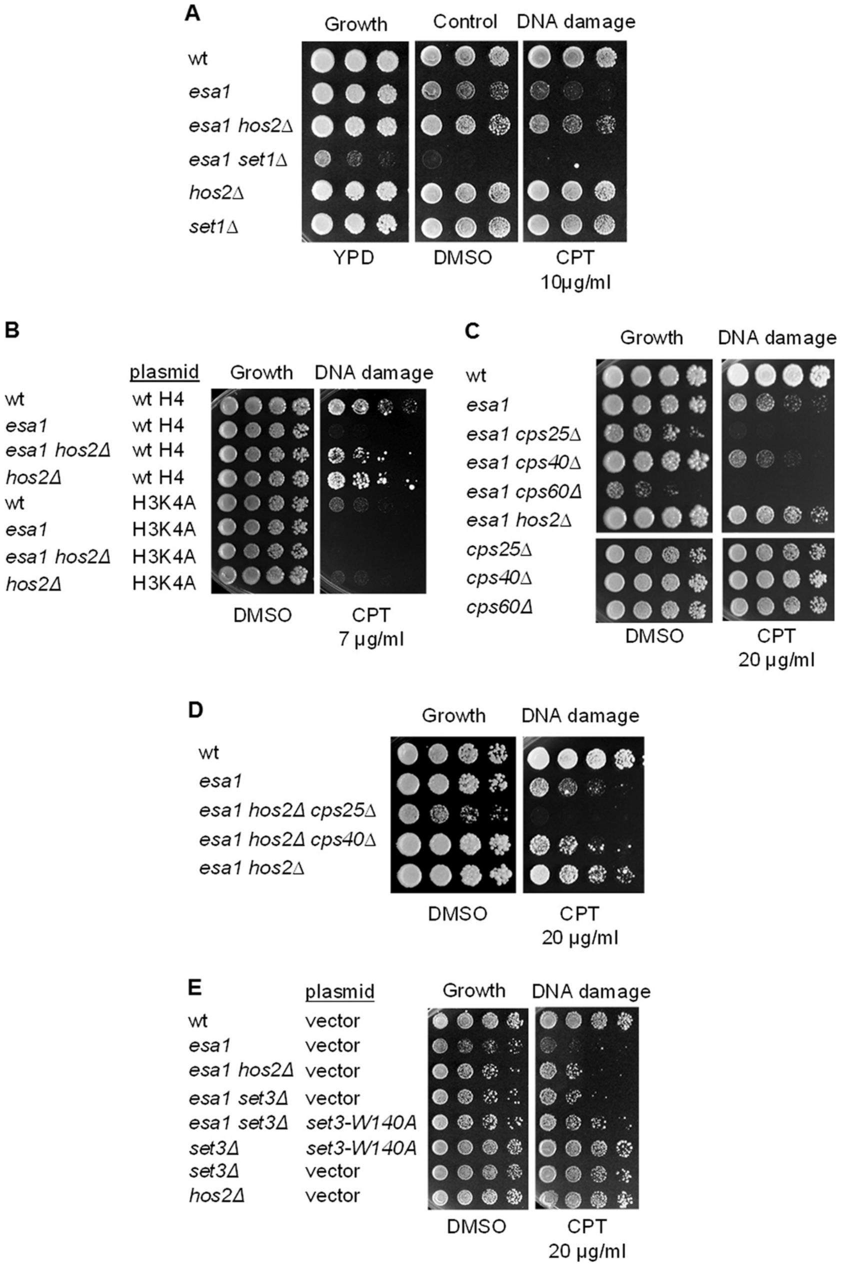

FIG 6 Loss of the methyl-binding domain of Set3 suppressed *esa1* DNA damage sensitivity. (A) The *esa1 set1*Δ strain was synthetically sick. The *esa1 set1*Δ strain grew slowly at 30°C on rich medium and was inviable when grown on 0.2% DMSO or 10 µg/ml of CPT. (B) Mutation of H3K4 to alanine disrupted suppression of *esa1* DNA damage sensitivity by *hos2*Δ, which was also sensitive to H3K4A. Shown are serial dilutions of wild-type (LPY14161), *esa1-414* (LPY14163), *esa1-414 hos2*Δ (LPY15906), and *hos2*Δ strains expressing wild-type histones from a plasmid; wild type (LPY21480), *esa1-414* (LPY21481), *esa1-414 hos2*Δ (LPY21482), and *hos2*Δ (LPY21483) strains expressing H3K4A from a plasmid. (C) *esa1* strains are synthetically sick when combined with COMPASS complex deletions promoting simultaneous loss of H3K4 di- and trimethylation. The following strains were assayed: wild-type (LPY6497), *esa1-531* (LPY14757), *esa1-531 cps25*Δ (LPY21498), *esa1-531 cps40*Δ (LPY21495), *esa1-531 cps60*Δ (LPY21503), *esa1-531 hos2*Δ (LPY14761), *cps25*Δ (LPY21499), *cps40*Δ (LPY21494), and *cps60*Δ (LPY21520) strains. (D) *hos2*Δ could not suppress *esa1* when *CPS25* or *CPS40* were also deleted. Strains tested included wild-type (LPY6497), *esa1-531* (LPY14757), *esa1-531 hos2*Δ *cps25*Δ (LPY21656), *esa1-531 hos2*Δ *cps40*Δ (LPY21661), and *esa1-531 hos2*Δ (LPY14761) strains. (E) Vector-transformed (pLP1358) wild-type, *esa1-531*, *esa1-531 hos2*Δ, *esa1-531 set3*Δ, *set3*Δ, and *hos2*Δ strains shown in Fig. 5B and Fig. S3 in the supplemental material were compared to *set3-W140* (PHD domain mutant, pLP3020)-transformed *esa1-531 set3*Δ and *set3*Δ strains.

independent, parallel pathways (69), and because COMPASS mutants cannot impair H3K4me2 without affecting H3K4me3, which was also shown to be important for suppression.

To evaluate the significance of binding of Set3C to the H3K4me2 mark in suppression, we used the *set3-PHD* domain mutant (*set3-W140A*) (40). This mutant retains Set3C integrity but loses the recognition of the H3K4me2 mark (40). We asked if a *set3*Δ strain transformed with a vector or with the *set3-W140A* allele could suppress CPT sensitivity in *esa1*. This *set3-PHD* mutant did suppress (Fig. 6E), validating a mechanism in which lo-

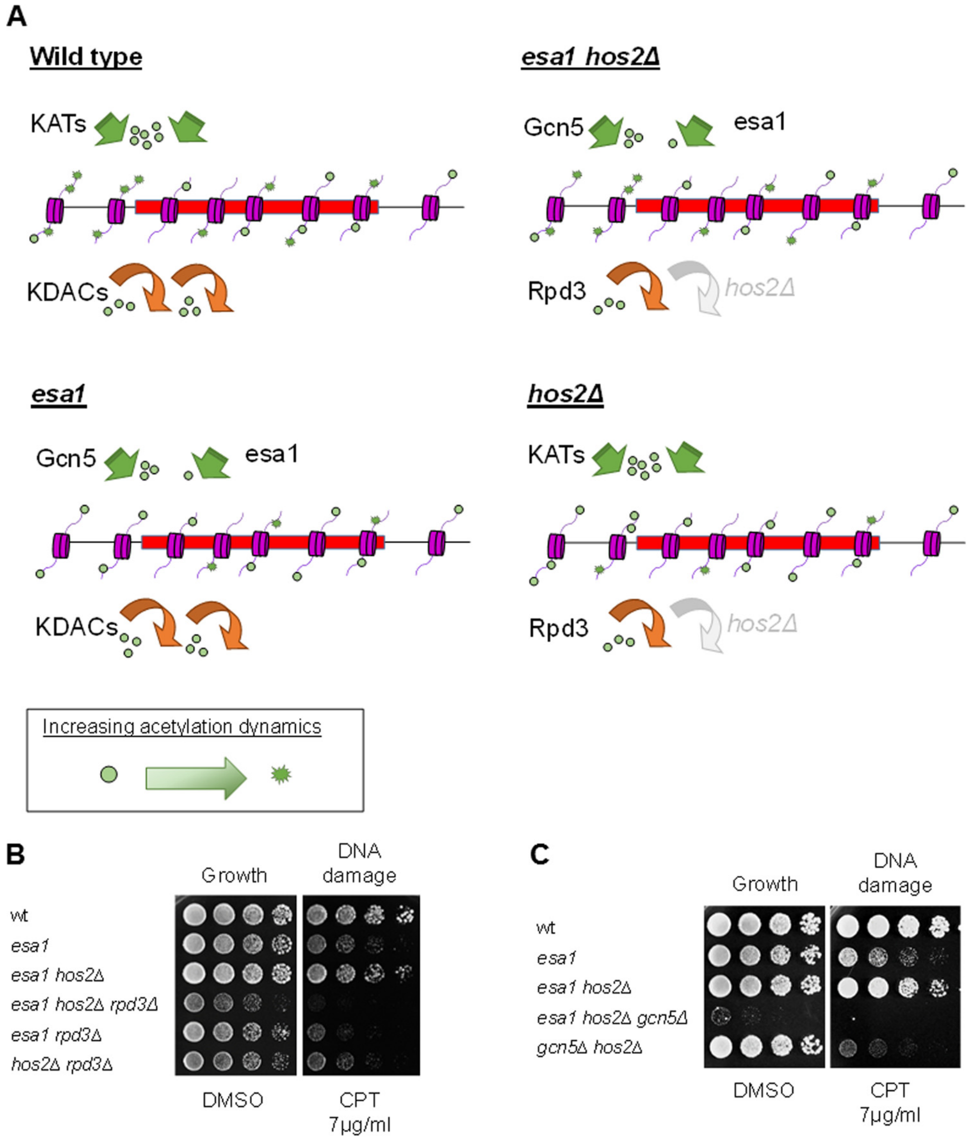

FIG 7 Potential impact of dynamic acetylation in regulation of gene expression upon DNA damage. (A) Wild-type and *esa1 hos2*Δ strains would maintain dynamic acetylation compared to *esa1* and *hos2*Δ strains that have impaired dynamics. Acetylation is depicted by green circles on histone tails. (B) *HOS2* deletion could not suppress *esa1* in the absence of *RPD3*. Wild-type (LPY6497), *esa1-531* (LPY14757), *esa1-531 hos2*Δ (LPY14761), *esa1-531 hos2*Δ *rpd3*Δ (LPY21428), *esa1-531 rpd3*Δ (LPY21450), and *hos2*Δ *rpd3*Δ (LPY21426) strains were plated on the indicated medium. (C) *HOS2* deletion could not suppress *esa1* in the absence of *GCN5*. Wild-type (LPY5), *esa1-414* (LPY21400), *esa1-414 hos2*Δ (LPY21401), *esa1-414 hos2*Δ *gcn5*Δ (LPY21468), and *gcn5*Δ *hos2*Δ (LPY21399) strains were plated as indicated. The *esa1-414 gcn5*Δ strain was not included in the plating, as it was lethal when having *esa1-414* expressed from a plasmid in the W303 background.

calized loss of Set3 and Hos2 recruitment suppressed the DNA damage sensitivity of *esa1* strains.

DISCUSSION

The essential acetyltransferase Esa1 and its human ortholog Tip60 have key roles in responding to DNA damage. Esa1 participates in induction of gene expression and is recruited to sites of DNA damage, where it promotes ligation of broken DNA ends. Further, because Esa1 is involved in regulating cell cycle progression, it may also have a role in establishing cell cycle delays necessary for repair.

The powerful tool of genetic suppression has provided insight into the function of Esa1 through identification of specific conditions that relieve *esa1* phenotypes. These include deletion of specific subunits of the Rpd3L and Rpd3S complexes (23, 34), as well as overexpression of the RNA binding protein Nab3 (14) and the amino acid biosynthetic protein Lys20 (33, 35). Each of these genetic manipulations suppresses a unique constellation of phenotypes of Esa1. For example, loss of Rpd3 rescues temperature sensitivity, silencing defects and diminished histone H4 acetylation (23), whereas *NAB3* overexpression suppresses *esa1*'s silencing defects and temperature sensitivity (14). In contrast to *LYS20*, neither *rpd3*Δ nor *NAB3* overexpression improves the DNA damage response of *esa1* mutants. In this work, we established that deletion of the deacetylase encoded by *HOS2* suppressed DNA damage sensitivity of *esa1*, underscoring the diverse and intricate interactions of the chromatin-modifying activities.

Suppression of *esa1* DNA damage sensitivity by *hos2*Δ correlated with enhanced acetylation of histone H4. A similar global increase in H4 acetylation correlates with suppression of a different set of phenotypes when the Rpd3L complex is removed. An important question, then, is this: why does enhanced global H4 acetylation in *esa1* cells rescue independent phenotypes depending on the deacetylase removed?

To begin to answer this question, it should be noted that the Rpd3L complex is targeted to promoter regions by the transcription factor subunits Ash1 and Ume6 (70). Through its PHD domain subunit, Pho23, Rpd3L is also capable of recognizing the H3K4me3 mark usually found at promoter regions. In contrast, Set3C binds genomic areas enriched with the H3K4me2 mark. Dimethylation of H3K4 is generally localized 5′ of ORFs (40); however, it can also replace H3K4me3 at specific promoters (38), including, for example, the promoter of *GRE2* (56). We propose that *RPD3* and *HOS2* deletions suppress different phenotypes of *esa1* because their loss promotes increased histone H4 acetylation at specific genomic areas or genes: loss of Rpd3L would promote increased acetylation at promoter regions, whereas loss of *HOS2* would lead to enhanced acetylation at ORFs and select promoters regulated by H3K4me2. Indeed, dynamic relocalization of Hos2 has been reported upon MMS treatment to facilitate formation of noncanonical repair foci (71), hypothesized to be transcriptional factories.

Suppression of the DNA damage sensitivity of *esa1* by *hos2*Δ could be mediated by the transcriptional response to damage or by direct recruitment of the complex to broken DNA ends. Because Hos2 was previously shown not to be recruited to areas enriched with Esa1 following induction of a single DSB by the HO endonuclease (7), it is likely that Hos2 opposes Esa1 through a different pathway.

We confirmed earlier results showing defective induction of *HUG1* in *hos2*Δ and *esa1* strains and tested other genes previously established as induced by DNA damage. The *esa1* strain had an impaired transcriptional response to damage. The gene expression profile of *hos2*Δ cells was also abnormal compared to that of the wild type (Fig. 4A). This is in agreement with an impaired DNA damage response of *hos2*Δ and with its proposed role in acetylation dynamics involved in gene induction (31). The *hos2*Δ strain is not sensitive to DNA damage, suggesting that high induction of a specific set of genes during repair, such as *HUG1*, may function as a protective transcriptional response.

Upon damage induction, the gene expression profile in the *esa1 hos2*Δ strain was distinct from that of *esa1*. This response remained distinct from that of the wild type, suggesting that in a manner similar to that in the *hos2*Δ strain, the combined response in *esa1 hos2*Δ cells may be sufficient to promote resistance to DNA damage. Further supporting this idea, gene expression in the damage-sensitive *esa1 rpd3*Δ strain proved similar to that in the *esa1* strain upon DNA damage. Additional insight into suppression of *esa1*'s defective response to damage will ultimately be obtained with global analyses correlating gene expression and histone H4 acetylation with Rpd3L and Set3C occupancy at promoter and coding regions upon damage.

In defining suppression of *esa1* by *hos2*Δ, we found that it could be mediated by removal of any Set3C subunit, with the strongest effects seen upon deletion of *HOS2*, *HOS4*, *SET3*, and *HST1*. Loss of Set3 and Hos2 disassemble Set3C, whereas deletion of *HOS4* leads to loss of Hst1 association with the complex, suggesting that both KDACs, Hos2 and Hst1, have roles in opposing Esa1 during DNA damage repair. Since Hst1 and Hos2 have both shared and specific targets (38), future studies will define how deletion of *HST1* affects *esa1* upon DNA damage. In agreement with the expression data, genetic dissection revealed that suppression of *esa1* by *hos2*Δ required chromosomal binding of Set3C to the H3K4me2 mark, a histone modification already implicated in cellular stress response.

Taking the results together, it appears that Esa1 and Hos2 oppose each other by promoting dynamic acetylation and deacetylation. In the absence of active Esa1 and Hos2, other HATs, such as Gcn5, and KDACs, such as Rpd3, can promote acetylation dynamics (Fig. 7A). In this scenario, the *esa1* and *hos2*Δ single mutant strains have impaired acetylation dynamics and gene regulation in response to DNA damage (Fig. 7A). The dynamics would be reestablished in the *esa1 hos2*Δ strain by promoting an adjusted transcriptional response to allow growth following damage. In support of this idea, *RPD3* and *GCN5* proved necessary for suppression of *esa1* by *HOS2* deletion (Fig. 7B and C). Because of the conserved nature of these regulators of acetylation, continued dissection of their functional interactions will contribute to a deeper understanding of their fundamental roles. Understanding how Esa1 participates in DNA damage repair will ultimately point to mechanisms defining the role of human Tip60 in cancer progression associated with genomic instability and DNA damage.

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